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Virally-Induced Upregulation of Heparan Sulfate on B Cells via the Action of Type I IFN

Nadine Jarousse,*1 Damian L. Trujillo,*1 Sarah Wilcox-Adelman,† and Laurent Coscoy*1

Cell surface heparan sulfate (HS) is an important coreceptor for many cytokines, chemokines, and growth factors. In this study, we report that splenic murine B cells express very little HS and that upon infection with either gammaherpesvirus (murine gammaherpesvirus 68) or betaherpesvirus (murine cytomegalovirus), HS is rapidly upregulated at the surface of B cells. HS upregulation was not observed in mice deficient for the type I IFN (IFN-I) receptor. Additionally, treatment of wild-type mice with the IFN-I inducer polyinosine polycytidylic acid triggered HS expression at the B cell surface. Similarly, incubation of purified splenic B cells with IFN-I, TLR ligands, or BCR stimulators ex vivo resulted in a drastic increase in HS surface expression. We found that IFN-I induced an increase in the surface expression of HS-modified syndecan 4 as well as that of an unidentified heparan sulfate proteoglycan. Finally, IFN-I treatment increased B cell responsiveness to APRIL, a cytokine involved in B cell survival and T cell-independent B cell responses. Enzymatic removal of HS from IFN-I–treated B cells inhibited APRIL. Altogether, our results indicate that upon herpesvirus infection in mice, HS is rapidly upregulated at the surface of B cells due to the action of IFN-I, potentially increasing B cell responsiveness to cytokines. Induction of HS expression at the B cell surface by stimulators of the innate immune response likely plays a key role in the development of a robust immune response.


HS is commonly described as a ubiquitously expressed glycosaminoglycan, but to date its expression and function have been examined mainly in adherent cells. Although many cytokines involved in immune function are known to bind HS (IL-2 to IL-8, IL-10, IL-12, IFN-γ, TNF-α, MIP-1β, APRIL, etc.) (2), little is known about the contribution of HS to lymphocyte biology. Although HS molecules were shown to play a role in IL-7–dependent B lymphopoiesis (8), the importance of HS chains at the surface of mature B cells is not clear. Intriguingly, HSPGs are expressed at certain stages of B lymphocyte differentiation. For example, syndecan 1 (CD138) is expressed on pre-B cells, absent in circulating and peripheral B lymphocytes, and re-expressed upon differentiation to the plasma cell stage (9). Syndecan 4 is expressed on bone-marrow B cells and most mature B cell subsets (10). Although the expression of syndecans is tightly regulated, their function in B cell biology are not defined.

We recently reported that B cell lines express little to no HS at their surface (11), which prompted us to examine the status of HS at the surface of naive splenic B cells. In this study, we report that naive splenic murine B cells express very little HS at their surface, but that HS expression was upregulated upon a type I IFN (IFN-I) response in vivo. This upregulation was essential for the signaling of APRIL, an HS-binding cytokine (12) involved in class switch recombination and B cell survival (13, 14). Attachment of HS to syndecan 4 and a 130-kDa HSPG accounts for the induction of HS on B cells. Altogether, our results suggest that in the context of an infection that triggers an IFN-I response, B cell responsiveness to cytokines and other HS-binding ligands might be amplified due to an upregulation of HS surface display.

Materials and Methods
Mice and viruses
C57BL/6 mice were obtained from The Jackson Laboratory. Mice deficient for the IFN-α receptor [IFNAR knockout (KO) mice] were a kind gift from Dr. Daniel Portnoy, University of California, Berkeley. Bone marrow from syndecan 4-deficient mice was a kind gift from Dr. Sarah Wilcox-Adelman. Experiments were conducted with 6- to 12-wk-old mice in accordance with institutional guidelines for animal care and use. Murine
gammaherpesvirus 68 (MHV68) was obtained from the American Type Culture Collection (VR-1465) and propagated on BHK-21 cells. Smith strain murine cytomegalovirus (MCMV; MW97.01) was a kind gift from Dr. David Raulet, University of California, Berkeley. MCMV was propagated on NIH3T3 cells. Virus-containing supernatants were cleared of cellular debris by low-speed centrifugation for 20 min. Virus was subsequently concentrated by centrifugation at 24,000 × g for 2 h and purified over a 30% sucrose cushion by centrifugation at 85,000 × g. Pelleted virus was resuspended in PBS and stored at −80°C. Viral titers were determined on NIH3T3 cells using a standard plaque assay. BHK-21 and NIH3T3 cells were grown in DMEM supplemented with 10% FBS and 100 units/ml penicillin and 100 μg/ml streptomycin.

Isolation and culture of splenic B cells

Splenocytes were isolated from C57BL/6 or IFNAR KO mice, and B cells were purified using the EasySep negative selection mouse B cell enrichment kit (Stem Cell Technologies). B cell purity was assessed by flow cytometry using anti-CD19 or anti-B220 Abs (≥98% purity). Cell viability was verified by propidium iodide or 7-aminocoumarin D staining (>99% viability). Cells were resuspended at a density of 2 × 10^6 cells/ml (except for APRIL assay; see below) in RPMI media containing 100 μM MEM nonessential amino acids, 55 μM 2-mercaptoethanol, 1 mM sodium pyruvate, and 10 mM HEPES. When indicated, reagents were added to the media: IFN-γ at 1000 U/ml unless indicated otherwise (PBL, IFN source), anti-IgM at 2 μg/ml (Jackson ImmunoResearch), type A and type B CpG oligonucleotides at 3 μM, and LPS at 1 μg/ml.

Flow cytometry

Splenocytes or purified splenic B cells were washed in PBS with 1% BSA and incubated with 1 μg/ml purified rat anti-mouse CD16/CD32 (Fc block; BD Pharamingen) at 4°C for 15 min, followed with an anti-IgM Ab used at 1:100 dilution (anti-IgM, F58-10E4; Seikagaku) for 30 min at 4°C. The isotype control was a mouse IgM (TEPC 183; Sigma). After two washes in PBS with 1% BSA, bound Ab was revealed by staining with an FITC- or PE-conjugated anti-mouse IgM (Igh-6a) mAb (BD Pharmingen). When staining cultured B cells, dead cells were excluded from the analysis by propidium iodide or 7-aminoactinomycin D staining.

Measure of APRIL-mediated IgA production

293T cells were transfected with either PCDNA3.1 (vector control) or a vector encoding soluble APRIL (a kind gift from Dr. Kimberley Fiona, University of Amsterdam, Amsterdam, The Netherlands) using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Two days later, supernatant was harvested from transfected 293T cells and diluted 1:2 in RPMI that included supplements and FBS. Diluted supernatant was used to resuspend purified splenic B cells (1 × 10^6 cells/ml). Then 2 × 10^4 cells/well (200 μl) were plated in a round-bottom 96-well plate. Cells were left untreated, treated with either IFN-β (45 U/ml) or with 3 units/ml of heparinase III (Sigma-Aldrich), and incubated with 1000 U/ml unless indicated otherwise (PBL, IFN source), or both simultaneously. Pelleted virus was used to resuspend purified splenic B cells (1 × 10^6 cells/ml). Then 2 × 10^4 cells/well (200 μl) were plated in a round-bottom 96-well plate. Cells were left untreated, treated with either IFN-β (45 U/ml) or with 3 units/ml of heparinase III (Sigma-Aldrich), or both simultaneously. Cells were cultured for 6 d. At days 2 and 4 postplating, an additional 0.6 U/well of heparinase was added to all heparinase-treated samples. At day 6 postplating, supernatant from wells was collected and added to ELISA plates coated with anti-IgA Ab (Southern Biotech). IgA production was measured with an alkaline phosphatase-conjugated anti-IgA Ab. Substrate conversion (pNpP, cat. no. N2770; Sigma) was monitored using a plate reader measuring at 405 nm. A standard curve using known concentrations of IgA was generated and used to determine IgA concentration (ng/ml) in the B cell supernatants.

Generation of bone marrow chimeras

Female C57BL/6 congenic mice (CD45.1) between 6 and 8 wk were lethally irradiated with 900 rad. Twenty-four hours later, the mice were injected i.v. with 5 × 10^8 bone marrow cells from syngeneic-deficient mice (C57BL/6 background; CD45.2^-). Ten weeks postinjection, reconstitution efficiency was checked by tail-vein bleeding.

Western blot and immunoprecipitation

Purified ex vivo B cells were treated or not with IFN-I, then were incubated with or without heparinase and chondroitinase (heparinase: cat. no. H8891-50UN, 10^7 cells/ml; chondroitinase: cat. no. C3667-10UN, 10^7 cells/ml, 0.005 U/ml; Sigma) for 3 h at 37°C. Cells were then washed and lysed prior to SDS-PAGE. For immunoprecipitation, lysate was preclarified with protein A/G beads (cat. no. sc2003; Santa Cruz Biotechnology) and then immunoprecipitated using an anti-syndecan 4 Ab (cat. no. ab24511; Abcam). The immunoprecipitate was washed prior to SDS-PAGE. After transferring to immobiline membrane (Thermo Fisher), the membrane was probed first with anti-HS (clone 3G10, cat. no. 370260-1; Seikagaku) and then with FRP-conjugated anti-mouse IgG. Western blots were developed using Super Signal West Pico Chemiluminescent Substrate (cat. no. 34080; Pierce Biotechnology), and exposed onto film.

Statistical analysis

All statistical results are expressed as mean ± SEM. Statistical analysis was performed using a nonparametric Mann–Whitney U test for comparison between two groups (GraphPad software). Differences were considered significant at p < 0.05.

Results

Naive B cells express low levels of surface HS

To determine the expression level of HS on naive murine B cells, splenocytes from C57BL/6 mice were isolated, stained with a commonly used anti-HS Ab (F58-10E4; Seikagaku), and analyzed by flow cytometry. B cells were identified using an anti-B220 Ab (similar results were obtained using an anti-CD19 Ab). The murine NIH3T3 fibroblast cell line was used as a positive control (Fig. 1). Similar to what we observed in B cell lines (11), we found that the level of HS expression on the surface of splenic B cells is very low or undetectable. Likewise, van der Voort et al. (15) reported that HS was hardly detectable at the surface of resting human tonsillar B cells. Thus, it appears that neither murine nor human naive B cells express a significant level of HS at their surface.

HS is upregulated at the B cell surface after herpesvirus infection in vivo

HS participates in cell surface binding of many cytokines involved in inflammation and also plays an important role in cell adhesion and migration (2). Notably, van der Voort et al. (15) showed that HS was upregulated at the surface of human tonsillar B cells upon activation in culture. Thus, we speculated that HS expression might be upregulated at the surface of B cells during an immune response in vivo, and this upregulation might be important for B cell function. To test whether HS is upregulated on B lymphocytes in the context of an infection in vivo, wild-type C57BL/6 mice were infected with MHV68 either by i.v. or i.p. infection. Mice were sacrificed at various time points postinfection, and splenocytes were examined for HS expression as

![FIGURE 1.](http://www.jimmunol.org/)
described earlier. HS is upregulated at the B cell surface as early as 12 h after MHV68 i.v. infection (Fig. 2A, left panel). Importantly, HS upregulation is observed on the entire B cell population (Fig. 2A, middle panel). The increase in HS surface expression is also detected after i.p. infection (Fig. 2A, right panel). Although HS induction on the entire B cell population as early as 12 h postinfection is unlikely to be the result of a direct interaction with MHV68, we verified that HS upregulation was also observed after infection with MCMV, a virus that does not target B cells. As shown in Fig. 2B, MCMV infection also triggers an increase in HS expression at the B cell surface after either i.v. (Fig. 2B, left panel) or i.p. (Fig. 2B, right panel) infection. In all cases, the entire B cell population exhibits enhanced HS expression, suggesting the possible action of a diffusible agent such as a cytokine.

**HS upregulation is mediated by IFN-I**

IFN-I constitutes the first line of host defense against viruses (16). IFN-I plays a critical role in controlling both the acute and latent phases of MHV68 infection (17, 18). To test whether IFN-I is involved in HS upregulation in B cells after MHV68 infection, IFNAR KO mice were infected with MHV68, and HS expression on B cells was examined at 36 h postinfection. Whereas HS was strongly induced on B cells from infected wild-type mice, the level of HS expression on B cells from infected IFNAR KO mice was close to background, comparable with that of control PBS-injected mice (Fig. 3A), indicating a role for IFN-I in HS upregulation during an in vivo immune response.

**To test further the role of IFN-I in HS induction, we used another model of IFN-I induction in the absence of viral infection. Polyinosine polycytidylic acid (poly I:C) is a dsRNA mimetic that is commonly used to trigger IFN-I production in vivo (19). Wild-type (wt) C57BL/6 mice were treated with poly I:C (i.v. injection), and HS expression was measured at the surface of splenic B cells. Enhanced HS expression was observed as early as 12 h after poly I:C injection (Fig. 3B, left panel). Notably, the induction of HS after poly I:C injection was observed only on B cells, as the non-B cell fraction of splenocytes showed no significant HS expression (Fig. 3B, right panel).

To test the direct effect of IFN-I on B cells, in the absence of other cell types or cytokines, splenic B cells were purified from wt mice and cultured in the presence of recombinant IFN-β for 24 h. Surface HS was examined by flow cytometry. Culturing purified B cells for 24 h, in the absence of IFN-I or any other stimulator, led to an upregulation of surface HS (Fig. 3C, media). Notably, IFN treatment results in a dramatic increase in HS expression over media alone (Fig. 3C, IFN-I). Importantly, the induction of HS on B cells is not due to HSPG binding TACI (Supplemental Fig. 1A), as IFN-I did not induce TACI on B cells. Altogether, our results indicate that IFN-I–mediated signaling triggers upregulation of HS expression at the surface of B cells and accounts for the upregulation observed in vivo upon MHV68 infection. Notably, the increased HS on B cells upon culturing (Fig. 3C, media) is also observed on B cells isolated from IFNAR KO mice (Fig. 3D, right panel), indicating that signals other than IFN-I can trigger HS to be upregulated in culture.
Activation of purified splenic B cells through the B cell Ag receptor (via an anti-IgM Ab) also results in an increase in HS expression over media alone (compare Fig. 4A and 4B), consistent with an earlier study on human tonsillar B cells (15). Similarly, stimulation of TLR4 with LPS (Fig. 4C), TLR9 with type A (Fig. 4D) or type B (Fig. 4E) CpG oligonucleotides leads to an upregulation of HS at the B cell surface. Although this upregulation of HS is not dependent on IFN-I signaling, IFN-I does potentiate HS induction as demonstrated by the lower amount of HS observed on purified IFNAR KO B cells treated with the above stimulatory ligands (Fig. 4, dashed lines). Altogether, our results indicate that a variety of stimuli normally present during the course of an infection, such as IFN-I, TLR ligands, or molecules capable of cross-linking BCRs, cause HS to be upregulated in B cells.

Syndecan 4 and a 130-kDa HSPG are modified with HS on B cells upon IFN-I treatment

We hypothesized that the increase in HS expression on B cells was due to an increase in expression of HSPGs. To investigate this possibility, we decided to examine the expression of all known HSPGs (syndecan 1–4, glypican 1–6, TGF-βR3, agrin, and CD44v3). Their expression in naive and IFN-I–treated B cells was examined by reverse transcription quantitative-PCR and surface
staining (when commercial Abs were available). We observed that whereas most HSPGs were expressed at extremely low levels or not expressed at all (data not shown), syndecan 4 was readily detectable. However, its surface expression was only slightly increased in B cells cultured in the presence of IFN-I compared with that in freshly isolated naive B cells (Fig. 5A). To determine if syndecan 4 is modified with HS, ex vivo and IFN-I–treated B cells were treated with or without heparinase (an enzyme that degrades HS). Cells were then lysed, and syndecan 4 was immunoprecipitated. This was followed by a Western blot for HS using the 3G10 Ab (Seikagaku), which recognizes a neo-epitope that is generated upon heparinase treatment. Fig. 5B shows that syndecan 4 from IFN-I–treated B cells, but not from naive B cells, is associated with HS (compare heparinase treated ex vivo and IFN-I treated lanes), indicating that syndecan 4 is modified with HS in response to IFN-I treatment.

To test if syndecan 4 is the sole HSPG that accounts for HS surface expression on B cells, we generated syndecan 4 KO bone marrow chimeras by i.v. injection of syndecan 4 KO bone marrow (CD45.2+) into lethally irradiated congenic wt mice (CD45.1+). Upon reconstitution, we confirmed that syndecan 4 was not expressed in CD45.2+ B cells by RT-PCR (data not shown). B cells were purified from wt and syndecan 4 KO bone marrow chimeras and treated in culture with IFN-I. Twenty-four hours later, HS expression on B cells was assessed by flow cytometry. Surprisingly, we observed that HS induction was not affected by the absence of syndecan 4 expression on B cells (Fig. 5C).

To take an unbiased approach in identifying HSPGs expressed on B cells, we performed a Western blot for HS on lysates isolated from ex vivo or IFN-I–treated B cells (using the 3G10 Ab). In agreement with our above results, HS expression was not detected on ex vivo B cells (Fig. 5D). However, in IFN-I–treated wt B cells, we observed three bands corresponding with the molecular mass of ~130, 55, and 37 kDa. Two of these bands (55 and 37 kDa) were absent in IFN-I–treated syndecan 4 KO B cells, suggesting that these two lower molecular mass bands correspond with syndecan 4. This is in agreement with the molecular mass of HS-modified syndecan 4 observed in Fig. 5B. The presence of a 130-kDa band in IFN-I–treated wt and syndecan 4-deficient B cells suggests that HS moieties are also carried by an additional high molecular mass HSPG. Notably, culturing of B cells in the absence of IFN-I induced only HS-modified syndecan 4 (Supplemental Fig. 2). Only when B cells were cultured in the presence of IFN-I, or isolated from a poly I:C-injected mouse, were both syndecan 4 and the 130-kDa HSPG induced. These data indicate that various stimuli can induce HS-modified syndecan 4, but IFN-I signaling induces HS modification of both syndecan 4 and the 130-kDa HSPG.

**FIGURE 5.** Syndecan 4 is modified with HS on B cells upon IFN-I treatment. A. Purified B cells from C57BL/6, either ex vivo (solid line) or treated with IFN-I for 24 h (dashed line), were stained for syndecan 4 and analyzed by flow cytometry. Isotype control is represented by shaded histogram. B. Purified B cells, either cultured for 24 h in the presence of IFN-I or isolated immediately ex vivo, were treated with or without heparinase III and chondroitinase before being lysed and immunoprecipitated for syndecan 4. Immunoprecipitates were then run on an SDS-PAGE gel before blotting for HS (3G10). Arrows indicate both syndecan 4 bands. Ab H chain (HC) and I chain (LC) are indicated. C. Splenic B cells were purified from either C57BL/6 (solid line) or syndecan 4+/- bone marrow chimeras (dashed line) and cultured in the presence of IFN-I. Twenty-four hours after IFN-I treatment, cells were harvested and stained for HS (populations gated on CD45.2+). Isotype control is represented by shaded histogram. D. Purified ex vivo or IFN-I–treated B cells were treated with or without heparinase and chondroitinase prior to lysis and Western blot for HS (clone 3G10; Seikagaku). The γ-axis for all histograms is percent of maximum. Numbers on blot indicate kDa molecular mass marker. Histograms and Western blot are representative experiments, using one to two mice, and were performed at least three times.
HS expression on B cells is essential for APRIL-mediated signaling

Given the role of HS in receptor signaling, we hypothesized that HS induction at the B cell surface increases responsiveness to HS-binding cytokines (2). To address this hypothesis, we analyzed the signaling of APRIL, a member of the TNF family that has been shown to bind HS (12, 20). APRIL promotes B cell survival and IgG to IgA isotype switching (13). We treated purified B cells with soluble APRIL (produced by transfection of 293T cells) or with control media (supernatant from pcDNA3.1-transfected 293T cells), in the presence or absence of IFN-I and heparinase either individually or concomitantly. We then assessed APRIL signaling by measuring secreted IgA. As shown in Fig. 6A, we found that heparinase-treated B cells produced less IgA than untreated controls. IFN-I treatment, which we show induces HS expression, increased APRIL-mediated IgA production. Simultaneous addition of heparinase and IFN-I drastically reduced APRIL-induced IgA production. Importantly, B cell responsiveness to APRIL, via HS upregulation, was enhanced upon IFN-I signaling, as IgA production was reduced in IFNAR KO B cells (Fig. 6B). Altogether, these results indicate that IFN-I-induced HS expression on B cells is important for in vitro APRIL responsiveness.

Discussion

In this study, we present evidence that HS expression is tightly regulated in mature B cells in vivo; whereas HS is barely detectable in naive splenic B cells, its expression was upregulated by the action of IFN-I in mice. Additionally, we observed that HS expression was induced upon stimulation of murine splenic B cells via TLR-dependent pathways as well as BCR stimulation. Importantly, we did not observe an increase in HS expression on the non-B cell population after viral infections or poly I:C injection (Fig. 3B). This does not appear to be due to an inability of these cells to express HS, as ex vivo activation of monocytes and CD4 T lymphocytes increases HS expression at the cell surface (21, 22). Altogether, these results indicate that HS is poorly expressed on naive B cells, but its expression can be upregulated on lymphocytes upon exposure to a variety of stimuli normally present during the course of an infection. To our knowledge, our study is the first to show that HS is upregulated on immune cells in vivo. Our results are likely to be applicable to other species: van der Voort et al. (15) demonstrated that activation of ex vivo B cells, via BCR or CD40, enhances HS expression on human tonsillar B cells. Such dynamic regulation of HS expression suggests that HS may play a critical role in regulating immune function upon infection.

We hypothesized that the induction of HS could be mediated through upregulation of HSPGs, increased synthesis of HS, or a combination of both. To investigate the mechanism of HS upregulation on B cells, we examined both transcriptional and surface expression (when commercial Abs were available) of all known HSPGs. Of all HSPGs assessed, only syndecan 4 was expressed at significant levels. Its expression was only slightly enhanced upon treatment of B cells with IFN-I (Fig. 5A). However, we found that the amount of HS associated with syndecan 4 was dramatically increased in B cells that had been exposed to IFN-I (Fig. 5B). Notably, we also detected the presence of a high molecular mass HSPG (around 130 kDa; Fig. 5D). As this HSPG was only induced upon IFN-I signaling (Supplemental Fig. 2), the identity of this HSPG may give clues as to the function of HS upregulation on B cells in response to IFN-I signaling. The tendency of HSPGs to oligomerize or to run above their predicted molecular mass (23) makes it difficult to identify the nature of this HSPG.

We were surprised to find that HS expression was similar in IFN-I–treated wt and syndecan 4 KO B cells (Fig. 5C) given that syndecan 4 is modified with HS upon IFN-I treatment (Fig. 5B, 5D). One possibility is that the contribution of syndecan 4 to HS surface display is minor compared with that of the 130-kDa HSPG. Another possible explanation is that the synthesis of the mature form of HS containing N-sulfated glucosamine residues, or its detection by the Ab (epitope detected by the 10E4 Ab used in our flow cytometry experiments) is limiting in B cells under conditions that trigger a high level of HS (such as ex vivo IFN-I treatment; Fig. 5C). In the absence of syndecan 4, there would thus be an increased amount of HS linked to the high molecular mass HSPG, leading to similar levels of overall surface HS in wt and syndecan 4 KO B cells.

IFN-I treatment induces an upregulation of HS modification on syndecan 4 molecules (Fig. 5B, 5D), suggesting that IFN-I acts on HS biosynthesis. There are multiple steps where this may occur, as HS biosynthesis involves numerous enzymes (24). Initially, the proteoglycan is modified with a tetrasaccharide linker to which HS will be extended. This process is initiated by the XYL1 enzyme family. Upon completion of the tetrasaccharide linker, the enzymes EXT2 and EXT3 perform the addition of the first N-acetyl glucosamine, thereby dictating that the chain will be HS and not chondroitin sulfate. This is followed by disaccharide chain polymerization by the exostosin enzymes EXT1 and EXT2. Finally, the disaccharide chain is heterogeneously deacetylated and sulfated by the NDST enzyme family. To determine if HS synthesis was upregulated, we assessed the expression of HS-synthesizing enzymes by reverse transcription quantitative-PCR in ex vivo and IFN-I–treated B cells. Although we did not see an increase in the mRNA levels of the HS-polymerizing enzymes (EXT1 and EXT2), we did see a 2-fold induction in EXT3, EXT1, XYL1, NDST1, and NDST2 (data not shown).
Intriguingly, we also observed a 10-fold decrease in EXTL1 (data not shown). Whether or not this modest collective increase in expression of these enzymes contributes to the observed increase in surface HS remains to be determined. It is also possible that HS-synthesizing enzymes are regulated posttranslationally, and this could contribute to the increased HS in IFN-I–treated B cells.

APRIL, a member of the TNF family of ligands, is implicated in B cell survival, isotype switching, and T cell–independent Ab responses (13, 14, 25). We have shown that IFN-I treatment of B cells increased IgA production in response to APRIL in an HS–dependent manner. We observed that upon culturing, HS expression at the surface of B cells spontaneously increases (Fig. 3C, media). This likely helps explain why APRIL signaling can be measured in cultured B cells even in the absence of IFN-I or other stimulators of HS expression (12).

In addition to regulating cytokine responsiveness, HS is involved in a broad variety of biological processes including cell adhesion and migration (2). It would be interesting to determine if HS upregulation in response to IFN-I production has a direct implication on other aspects of B cell biology. For example, during an immune response, lymphocyte egress from lymphoid organs is temporarily shut down in response to several mediators including IFN-I. The role of IFN-I on lymphocyte retention is explained in part by inhibition of sphingosine 1-phosphate receptor-1 by CD69 (26). Because HS is involved in cell adhesion, its upregulation at the B cell surface might be another key factor involved in IFN-I–mediated inhibition of B cell egress from lymphoid organs. Similarly, it will be interesting to determine whether the presence of HS on B cells affects B cell movement and trafficking in secondary lymphoid organs and, ultimately, the ability of B cells to encounter and capture Ag as well as interact with T cells.

Viral infections are associated with a rapid and massive polyclonal B cell activation (27–30). This early activation is thought to contribute to the production of Abs, the induction of memory B cells, and the retention of B cells in lymphoid organs. IFN-I receptor deficiency on B cells has a profound impact on the quality and the magnitude of the early B cell response after influenza infection (28). Notably, groups have suggested that IFN-I signaling in B cells in vivo dramatically enhances the Ab response to Ags that are of low immunogenicity, such as chicken γ globulin (31, 32). Because of the pleiotropic effects of IFN-I, it is difficult to ascertain the precise mechanism by which IFN-I potentiates the B cell response. However, it is tempting to hypothesize that the induction of HS on B cells may be part of early B cell activation, and this contributes to an increased Ab response. Given the number of HS-binding cytokines that have immuno-modulatory activity (IL-2 to IL-8, IL-10, IL-12, IFN-γ, TNF-α, MIP-1β, APRIL (2)), HS induction may prove critical to B cell responses.

Reijmers et al. (33) recently published a report showing that mice whose lymphocytes are impaired in their ability to produce functional HS display decreased B cell numbers and diminished B cell function. These findings differ from those of Garner et al. (34), who found that mice lacking HS specifically on B cells displayed only slight changes in lymphocyte development and function. These discrepancies are likely to be explained by the different systems employed. Importantly, most assays done in both the Reijmers et al. and the Garner et al. studies were done in conditions that would not induce the high levels of HS we observed. Thus, it would be of interest to determine how IFN-I–, TLR–, or BCR-mediated induction of HS would affect B cell function in their experimental system.

Altogether, our results suggest that IFN-I–mediated regulation of HS expression serves as a novel mechanism to increase cytokine responsiveness of B cells upon viral infection (and thus the regulation of the Ab responses). Furthermore, if IFN-I–mediated HS induction on B cells enhances their responsiveness, this may have implications in the understanding of autoimmune disorders with a B cell component. For example, systemic lupus erythematosus, an autoimmune disorder characterized by elevated IFN-I and autoreactive B cells (35), may be better understood as a result of the work presented in this study.

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Disclosures

The authors have no financial conflicts of interest.

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